Puccinia punctiformis Affects Growth and Reproduction of Canada Thistle (*Cirsium arvense*)[1]

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Abstract. In growth chamber research, infection by *Puccinia punctiformis,* a rust fungus, reduced flowering and vegetative reproduction in Canada thistle. Disease symptoms were greatest when Canada thistle roots were stored at 5, 10, or 15 C following inoculation. Exposure of roots to different temperatures before inoculation did not affect disease. All Canada thistle clones tested, regardless of gender became diseased although there were differences in susceptibility to *P. punctiformis.* **Nomenclature:** Canada thistle, *Cirsium arvense* (L.) Scop. #[3]CIRAR.
Additional index words: Biological control, plant pathogen, thistle rust, CIRAR.

INTRODUCTION

Canada thistle is a noxious perennial weed that reproduces by seed and vegetatively by root buds (5, 15). Canada thistle seed in crop seedlots probably remains the chief means of dispersal to new localities (9, 11). Once Canada thistle has infested a new locality, vegetative reproduction is most likely responsible for dispersal that occurs within the area (1, 9, 12). Salisbury (16) reported that the radial growth of one interconnected clonal unit can spread up to 12 m in one growing season.

Cultivation segments Canada thistle root systems and increases the number of vegetative propagules (9, 12, 15). Root fragments as small as 10 to 12 mm in length produce aerial shoots (9, 12, 14). In addition, single herbicide applications do not provide long-term control due to the difficulty in killing the root system, which can survive even though the shoots have been destroyed (5, 12, 15). New technologies are necessary for effective, environmentally acceptable, and economical control of Canada thistle. A rust fungus, *Puccinia punctiformis* (F. Strauss) Rohl, may be useful as a biological control agent against Canada thistle (6).

Puccinia punctiformis (syn. *P. obtegens, P. suaveolens*) is an obligate parasite that is specific to Canada thistle (3, 4). This pathogen causes etiolation of systemically infected shoots and eventual necrosis of the leaves and stems. Teliospores represent a dormant overwintering stage of the fungus. Under favorable environmental conditions the teliospore germinates, produces a basidium bearing up to four basidiospores, each of which may germinate to infect adventitious root buds of the thistle plant. The mycelium infects new secondary shoots that emerge from roots. Orange spermagonia, followed by brown aecia are produced systemically on the abaxial leaf surfaces. Aeciospores, very similar to urediniospores, and teliospores are produced. Early signs of visible infection are the orange-colored spermagonia and a strong sweet fragrance (3). Aeciospores can infect nearby Canada thistle leaves, resulting in the production of uredinia, in which urediniospores and teliospores are produced. Urediniospores may overwinter in senesced leaves (3). Teliospores may overwinter in the soil and infect root buds the following growing season (7).

French and Lightfield (7) found that germination of *P. punctiformis* teliospores in a petri dish varied with temperature. Successful control of Canada thistle by *P. punctiformis* requires improved knowledge of the environmental constraints on infection. Turner et al. (18) found differences in disease incidence of *P. obtegens* among genotypes of Canada thistle. However, quantitative differences of intraspecific susceptibility of Canada thistle to *P. punctiformis* are not known.

Canada thistle is an imperfectly dioecious species (10, 11, 12). In most instances, pistillate flowers that produce only seed are borne on one plant, while staminate flowers that produce only pollen are borne on another plant. On occasion, however, florets on staminate plants bear both

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821-3133.

pollen and viable seed, thus indicating imperfect dioecism (10, 11, 12). It is known that systemically infected Canada thistle shoots contain higher concentrations of auxins and gibberellins than noninfected shoots (2). Hormonal changes may affect floral expression and gender (13). The effect of infection of Canada thistle by *P. punctiformis* on flowering in male or female plants is unknown.

The objectives of this research were to identify pre-inoculation and post-inoculation temperatures that promote disease by *P. punctiformis* in Canada thistle; to determine the effect of disease on sexual and asexual reproduction in Canada thistle; and to establish whether responses to disease differ due to gender or clone of Canada thistle.

MATERIALS AND METHODS

Effect of post-inoculation temperature on disease by *P. punctiformis* in Canada thistle of mixed genotypes. Roots of Canada thistle, originally collected at Ft. Detrick, Frederick, MD, were cut into 3- to 5-cm segments and pooled. Root buds were inoculated with teliospores of *Puccinia punctiformis* (7). Ten µl of a suspension of 6 mg of teliospores in 4 ml of water were applied to each root bud with a 3-mm loop. Each segment was planted 1 cm deep in pasteurized soil in a 10-cm clay pot. Pots were bottom-watered to avoid dislodging teliospores from the root buds.

Twenty of the inoculated root segments and five controls were placed in each of six incubators. Each of the incubators was maintained for a 3-wk period at one of the following constant temperatures: 5, 10, 15, 20, 25, and 30 C. A 14-h photoperiod with light intensity of 25 $\mu E/m^2/s$ was provided in each incubator to reduce etiolation. After the temperature treatment, the plants were removed from the incubators and placed in a growth chamber with a 14-h photoperiod (185 $\mu E/m^2/s$) and a 25 ± 2 C temperature. Plant heights and disease were measured at 2-wk intervals for five weeks and then monthly for two additional months. Disease was determined as the appearance of chlorosis, etiolation, and orange spermagonia.

This experiment was arranged in a completely randomized design. Five inoculated plants were grouped together based on position in a growth chamber. Four groups served as the basis for estimating expression of percent disease. The data were analyzed utilizing Pearson correlation coefficients and analysis of variance with mean separation based on the Fisher's protected least significant difference (17).

Effect of post-inoculation temperature on disease by *P. punctiformis* in three female and three male clones of Canada thistle. The experimental plant material used consisted of three female and three male Canada thistle clones (different from those used in the first experiment) that had been collected at Ft. Detrick, Frederick, MD, and vegetatively propagated by root cuttings. Roots were cut into 3 to 5 cm segments. Eighteen root segments per clone for each of the three male and the three female clones were inoculated by spraying (DeVilbiss laboratory sprayer) with 54.3 mg of teliospores in 50 ml of isopentane. Isopentane evaporated in less than 1 min and dispersed spores evenly over the roots with minimal wetting of the surface. In previous work isopentane alone was biologically inert and did not affect spore germination (8). Each root segment was potted in a 10-cm clay pot in pasteurized soil. Three potted root segments per clone were placed in each of six incubators for the post-inoculation temperature treatment, as described in the first experiment. Subsequent treatments and variables measured were the same as described in the first experiment. The data were analyzed for effect of temperature and clone using analysis of variance with mean separation based on Fisher's protected least significant difference and correlation and regression analyses.

Effect of pre-inoculation and post-inoculation temperature on disease by *P. punctiformis* in three female and three male clones of Canada thistle. Plants from the same clones utilized in the previous experiment were used for this experiment. Plants were vegetatively propagated in 10-cm clay pots and grown for 10 wk in a greenhouse before the pre-inoculation temperature treatment. Twelve plants per clone for each of the three female and three male clones were exposed to one of two pre-inoculation temperatures, 10 C or 20 C, for a 2-wk period. Ten and 20 C were selected based on the effect that these temperatures had in the second experiment. Roots were cut into 5-cm segments, each with a discernible root bud. One-half of the root segments, twelve per clone, were inoculated with *Puccinia punctiformis* teliospores, using the wire loop described previously. The remaining root segments were not inoculated. Each segment was planted 1 cm deep in pasteurized soil in a 10-cm clay pot. Six inoculated and six noninoculated segments from each clone were exposed to one of two post-inoculation temperatures, 10 C or 20 C, for a 3-wk period. Supplemental lighting reduced etiolation. At the end of the 3-wk period, three inoculated and

three noninoculated root segments from each clone had been exposed to the following preinoculation/post-inoculation temperature combinations: 10/10, 10/20, 20/10, and 20/20 C.

After the post-inoculation temperature treatment, all plants were moved to the same greenhouse. Weekly height measurements were made for a 14-wk period. The date of disease (i.e., appearance of symptoms), the number of diseased shoots, the total number of shoots, the date of flowering, and the total number and gender of flowers were recorded. At the time of harvest, the number of root buds were counted and shoots and roots were dried and weighed.

The experimental design was a four-factor factorial. The data were analyzed utilizing Pearson correlation coefficients and analysis of variance with mean separation based on the Fisher's protected least significant difference at P = 0.05.

Table 1. Effect of inoculation and 3-wk post-inoculation temperature on disease and growth of Canada thistle root cuttings inoculated with teliospores of *Puccinia punctiformis* and grown 3 mo in a growth chamber.

Main treatment effects[a]	Response after 3 mo	
	Height	Plants diseased
		%
Post-inoculation temperature, C		
5	18.2 bc	32 a
10	19.6 bc	52 a
15	19.4 bc	36 a
20	26.4 a	8 b
25	17.8 c	4 b
30	23.3 ab	0 b
Inoculation		
Inoculated	21.6 a	28 a
Not inoculated	17.7 b	0 a

[a]Within each column and main effect, means followed by same letter do not differ at the 0.05 level according to Fisher's protected LSD test (N = 20).

RESULTS AND DISCUSSION

Effect of post-inoculation temperature on disease by *P. punctiformis* in Canada thistle of mixed genotype. The greatest levels of disease occurred at 10 C (Table 1). Temperatures 5, 10, and 15 C produced significantly greater disease percentage than the remaining three temperatures: 20, 25, and 30 C. Lower temperatures may have enhanced germination of *P. punctiformis* teliospore and subsequent infection for several reasons. Temperatures of 10, 15, and 5 C decreased growth of Canada thistle root buds while teliospores still germinated. French and Lightfield (7) reported that maximum germination of *P. punctiformis* teliospores occurred below 20 C. Thus, the low temperatures may have suppressed root bud growth and enhanced teliospore germination and subsequent infection by *P. punctiformis.*

For the first 8 wk of growth, shoot height for Canada thistle of mixed genotypes was inversely correlated with disease (data not shown). It is possible that early in the infection process the fungus competes with the growing root bud for nutrients and water. The negative correlation decreased with time and by week 16, significantly positive correlations existed between height and percent disease. Inoculated plants were taller than noninoculated plants. The largest difference between inoculated and noninoculated plants occur at 10 C post-inoculation temperature, the temperature which produced the largest symptom expression of disease (Table 1).

The elongation of infected shoots (Table 1) was probably due to a hormonal imbalance, such as increased gibberellin levels, as noted by Bailiss and Wilson (2). The height-growth effect was confined to the diseased shoots and not generalized to the whole plant. That is, on the same plant, the diseased shoots were taller than the non-diseased shoots. Non-diseased shoots appeared normal. In addition, leaves on the diseased secondary shoots were narrower and sparser than on the non-diseased primary shoots.

Effect of post-inoculation temperature on disease by *P. punctiformis* in three female and three male clones of Canada thistle. A significant relationship was found between percent disease and post-inoculation temperature: Number of diseased plants (%) = 0.61 + 3.17 Temp – 0.11 Temp^2 (r^2 = 0.59). The number of diseased plants increased quickly as post-inoculation temperature increased from 5 to 10 C. Disease then decreased gradually as post-inoculation temperature continued to increase up to 20 C. French and Lightfield (7) reported an optimal temperature range of 16–20 C for teliospore germination on a 1% agar petri plate thermo-gradient system. They observed no germination below 8 C, after a 7-d incubation period. Evidently the optimum temperature range for disease, i.e., teliospore to basidium to basidiospore and subsequent infection of the thistle plant, does not coincide exactly with optimum temperature for teliospore germination. It is possible that the different propagules in the infection process may have different optimal temperatures.

There was no significant effect of clone or gender on

Table 2. Correlation between height and percent disease in six clones of Canada thistle inoculated with *Puccinia punctiformis.*

Time of height measurements[a]	Time of percent disease measurements, wk		
	8	12	16
wk	Pearson correlation coefficients		
2	-0.19[b]*	-0.22*	-0.24*
4	-0.20*	-0.23*	-0.26*
6	-0.05	-0.08	-0.11
8	0.08	0.10	0.05
12	0.27*	0.28*	0.24*
16	0.29*	0.30*	0.30*

[a]Number of weeks in a growth chamber.

[b]Designates a Pearson correlation coefficient between shoot length and percentage of disease that is significant at the 0.10 level of probability.

disease (data not shown). Temperature did not significantly affect height (data not shown).

As in the previous experiment, shoot height was inversely correlated with disease during the first six weeks of growth (Table 2). However, the negative correlation decreased with time and by week 12 of growth, significantly positive correlations existed between height and percent disease.

Effect of pre-inoculation and post-inoculation temperature on disease by *P. punctiformis* in three female and three male clones of Canada thistle. Temperature combinations of 20 C, pre- and 10 C post-inoculation temperature (hereafter pre- and post-inoculation temperature treatments will be referred to by numerical combination, e.g., 20/10) and 10/20 yielded the greatest percent disease and the smallest percent disease, respectively (Table 3). The temperature combination 20/10 verified that a post-inoculation temperature of 10 C enhances disease. The pre-inoculation temperature of 20 C likely stimulated metabolism of root buds, increasing the production of the root-stimulatory compound isolated by French et al. (6) and enhanced teliospore germination and infection.

Clones F3 and M3 were the only clones to show disease at all temperature combinations (data not shown). Clone F1 was the least susceptible to disease with only 8% of the plants being diseased (Table 3). These results suggest that there may be some variability in rust susceptibility of the genotypes of the Canada thistle tested. Turner et al. (18) also found some differences among Canada thistle ecotypes to disease by *P. obtegens* (*P. punctiformis*). In general, however, there was little evidence for differences in Canada thistle susceptibility due to clone or gender.

Table 3. Effect of inoculation, pre- and post-inoculation temperatures (for 2 and 3 wk, respectively), and clones on disease and growth of Canada thistle root cuttings inoculated with teliospores of *Puccinia punctiformis* and grown for 14 wk in a greenhouse.

Main treatment effects[a]	Plants diseased	Response after 14 wk			
		Shoot dry weight		Root dry weight	
	%	g			
Inoculation					
Inoculated	38 a	5.8 a		1.7 b	
Not inoculated	0 b	6.9 a		2.6 a	
		Inoculated	Not inoculated	Inoculated	Not inoculated
Pre- and post-inoculation temperatures, C					
10/10	33 ab	3.6 c	6.0 ab	0.7 b	1.9 b
10/20	17 b	5.0 bc	4.9 b	1.4 b	1.9 b
20/10	61 a	6.1 b	8.4 a	1.4 b	3.4 a
20/20	39 ab	8.4 a	8.4 a	3.2 a	3.0 ab
Clone					
F1	8 c	7.3 ab	7.9 ab	2.4 ab	3.0 ab
F2	25 bc	8.6 a	8.4 a	3.2 a	3.5 a
F3	33 abc	3.7 cd	5.2 bc	0.6 c	2.1 b
M1	33 abc	2.9 d	4.5 c	1.3 bc	1.3 b
M2	58 ab	5.7 bc	8.3 ab	1.1 c	2.8 a
M3	67 a	6.8 ab	7.2 abc	1.6 bc	2.5 a

[a]Temperature treatment numbers are pre-inoculation/post-inoculation temperatures. Clones designated F are female and M are male. There were three female and three male clones. Within a column and main effect, means followed by the same letter do not differ at the 0.05 level according to Fisher's protected LSD test.

Table 4. Effect of inoculation, pre- and post-inoculation temperatures (for 2 and 3 wk, respectively), and clones on survival and reproduction of Canada thistle root cuttings inoculated with teliospores of *Puccinia punctiformis* and grown for 14 wk in a greenhouse.

Main treatment effects[a]	Survival		Response after 14 wk: Flower		Response after 14 wk: Root shoots	
	%		no.			
Inoculation						
Inoculated	62 b		0.6 b		2.6 b	
Not inoculated	94 a		1.6 a		5.0 a	
	Inoculated	Not inoculated	Inoculated	Not inoculated	Inoculated	Not inoculated
Pre- and post-inoculation temperatures, C						
10/10	50	100	0.3	0.3 b	0.9 b	4.9
10/20	83	90	0.3	1.5 b	3.2 ab	5.0
20/10	57	100	0.7	1.3 b	1.4 b	6.0
20/20	59	87	1.2	3.3 a	5.1 a	3.9
	ns	ns	ns			
Clone						
F1	78	100	2.1 a	1.6 bc	3.1	3.2 b
F2	54	100	0.8 b	1.5 bc	3.8	6.4 ab
F3	83	100	0 b	0.1 c	0.3	4.3 b
M1	50	100	0 b	1.0 bc	2.8	4.0 b
M2	40	80	0.9 ab	3.6 a	3.3	8.5 a
M3	73	100	0 b	1.9 b	2.5	3.3 b
	ns	ns			ns	

[a]Temperature treatment numbers are pre-inoculation/post-inoculation temperatures. Clones designated F are female and M are male. Within a column and main effect, means followed by the same letter do not differ at the 0.05 level according to Fisher's protected LSD test. A column with "ns" indicates no significant difference due to treatment.

This experiment confirmed the findings of French and Lightfield (7) that infection by *P. punctiformis* usually precludes flowering. Only two out of a total of 26 diseased surviving plants produced flowers (data not shown). Perhaps more importantly, no diseased shoots produced flowers. Only non-diseased shoots on diseased plants were able to flower. Nutrient limitations or hormonal imbalances resulting from infection may account for reduced flowering.

Disease caused by *P. punctiformis* affects both vegetative propagation and sexual reproduction in Canada thistle by severely restricting, and in most cases preventing flowering and by reducing root bud production (Table 4). In spite of poor vigor demonstrated by the spindly, diseased shoots, shoot dry weight was not significantly affected by the disease. However, root dry weight was reduced by disease (Table 3). Evidently, patterns of assimilate partitioning were disrupted by disease which caused the reduction in root biomass. The physiological disruptions in flowering and growth may diminish the competitiveness of Canada thistle in the field.

Shoot height was positively correlated with the number of diseased shoots at weeks 7 and 14 (Table 5). The number of flowers, the number of root buds and the root dry weight were inversely correlated with the number of diseased shoots. The time of symptom appearance was not correlated with the growth and flowering variables measured. These results coincide with the first two experiments and also suggest that rapid appearance of infection symptoms

Table 5. Pearson correlation coefficients comparing time to disease symptoms of *Puccinia punctiformis* and number of infected shoots of Canada thistle with selected variables.

Selected variables	Time to disease, wk	Number of diseased shoots
	Pearson correlation coefficients	
Height at week 7	–0.16	0.34[a]*
Number of shoots at week 7	0.06	0.49*
Height at week 14	–0.05	0.14*
Number of shoots at week 14	–0.05	0.03
Number of flowers	0.13	–0.18*
Time to flowering	1.00	–0.07
Number of root buds	–0.18	–0.29*
Shoot dry weight	0.19	–0.02
Root dry weight	–0.04	–0.23*

[a]Designates a Pearson correlation coefficient that is significant at the 0.10 level of probability. No infected shoots flowered. Only two shoots on diseased plants flowered, and those shoots did not show sign of disease.

is not crucial to reduce growth of Canada thistle by *P. punctiformis*.

Inoculating Canada thistle roots with *P. punctiformis* and planting them may provide an effective way to grow inoculum in the greenhouse or to increase disease incidence and to manage stands of Canada thistle in the field. Good disease development of Canada thistle can be attained by applying teliospores to root buds and holding the inoculated root at 10 C for 3 wk. In these experiments the pre-inoculation/post-inoculation temperature combination 20/10 yielded the most disease by *P. punctiformis* and reduced flowering, vegetative propagation, and root biomass of Canada thistle. Field research is underway to determine the viability of this approach to use *P. punctiformis* as a biocontrol agent on Canada thistle.

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